

SODIUM PROPIONATE IN THE DEVELOPING  
CHICKEN EMBRYO

Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing  
Chicken Embryo-Report of the in-house investigations of Sodium Propionate in the  
developing chicken embryo 3/1/78

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# MEMORANDUM

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-335

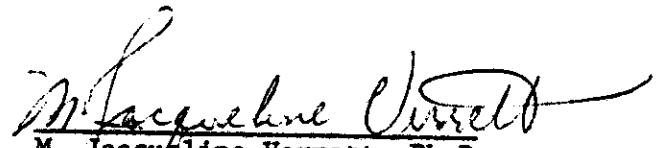
DATE: March 1, 1978

THRU: HFF-150 \_\_\_\_\_

FROM : Supervisory Chemist  
Whole Animal Toxicity Branch (HFF-155)

SUBJECT: Investigation of the Toxic and Teratogenic Effects  
of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the inhouse investigations of Sodium  
Propionate in the developing chicken embryo.

  
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Investigations of the Toxic and Teratogenic Effects of  
GRAS Substances to the Developing Chicken  
Embryo: Sodium Propionate

Protocol:

Sodium Propionate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch.

Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 gave the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

#### Discussion:

Sodium propionate showed moderate toxicity under the four conditions of test. The LD<sub>50</sub>s calculated were as follows:

Air Cell at 0 Hours: 134.14 mg/kg (6.71 mg/egg)  
Air Cell at 96 Hours: 84.49 mg/kg (4.22 mg/egg)  
Yolk at 0 Hours: 64.0 mg/kg (3.2 mg/egg)  
Yolk at 96 Hours: Not Calculated

Administration via the air cell resulted in a linear relationship between dose and abnormalities for both times of administration; however only at the highest test levels were the incidences of abnormalities significantly higher ( $p \leq 0.05$ ) than those in the solvent. The LC<sub>50</sub>'s for embryos having one or more abnormalities, and two or more abnormalities are indicated at the bottom of Tables 1 and 2. The most predominant abnormality observed was cleft palate, although these embryos frequently had other abnormalities as well. Yolk treatment did not result in any increase in serious abnormalities. Sodium propionate was teratogenic when administered via the air cell at both 0 and 96 hours of incubation.

1. Sodium Propionate, Pfizer Chemical Co., New York, NY Lot #73414
2. McLaughlin, J., Jr., Marliac, J.P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol 5, 760-770

3. Verrett, M. J., Marliac, J. P., and McLaughlin, J., Jr., (1964)  
JAOAC 47, 1002-1006
4. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press,  
Cambridge, Appendix I.

Sodium Propionate  
Air Cell at 0 Hours

Table 1

mg/egg	Dose		Number of Eggs	** Percent Mortality	Percent Abnormal	
	mg/kg				Total***	Structural
10.00	200.00		118	92.37*	32.20*	22.03*
5.00	100.00		120	49.16	5.00	1.66
2.50	50.00		120	34.16	6.66	1.66
1.250	25.00		120	35.00	2.50	2.50
0.50	10.00		120	28.33	0.83	0.00
Water			115	39.13	1.73	0.00
Control			332	9.33	1.50	1.20

\*Significantly different from solvent  $p \leq 0.05$

\*\*LD<sub>50</sub> 134.140 mg/kg (6.707 mg/egg)

\*\*\*LC<sub>50</sub> 386.343 mg/kg (19.3 mg/egg) one or more abnormalities

LC<sub>50</sub> 5673 mg/kg ( $>280$  mg/egg) two or more abnormalities

Sodium Propionate  
Air Cell at 96 Hours

Table 2

Dose mg/egg	mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal	
				Total ***	Structural
5.00	100.00	115	73.91*	13.91*	9.56*
2.50	50.00	115	26.08	3.47	2.60
1.250	25.00	115	28.69	6.08	1.73
0.6250	12.50	115	21.73	2.60	1.73
0.250	5.00	115	29.56	2.60	0.86
Water		120	23.33	2.50	1.66
Control		332	9.33	1.50	1.20

\*Significantly different from solvent  $p \leq 0.05$

\*\*LD<sub>50</sub> 84.494 mg/kg (4.224 mg/egg)

\*\*\*LC<sub>50</sub> 4381 mg/kg ( $> 200$  mg/egg) one or more abnormalities  
LC<sub>50</sub> 617 mg/kg (31 mg/egg) two or more abnormalities

Sodium Propionate  
Yolk at 0 Hours

Table 3

mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal	
				Total	Structural
10.00	200.00	120	89.16*	0.00	0.00
5.00	100.00	120	82.50*	0.83	0.83
2.50	50.00	120	75.83	1.66	0.00
1.250	25.00	119	69.74	4.20	1.68
0.50	10.00	120	70.00	4.16	2.50
Water		115	65.21	0.86	0.00
Control		332	9.33	1.50	1.20

\*Significantly different from solvent  $p \leq 0.05$

\*\*LD<sub>50</sub> 63.998 mg/kg (3.2 mg/egg)



Sodium Propionate  
Yolk at 96 Hours

Table 4

Dose mg/egg	mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal	
				Total	Structural
5.00	100.00	120	49.16	5.83*	2.50
2.50	50.00	120	58.33*	3.33	1.66
1.250	25.00	120	42.50	1.66	1.66
0.6250	12.50	119	44.53	2.52	1.68
0.250	5.00	120	36.66	3.33	2.50
Water		119	37.81	0.00	0.00
Control		332	9.33	1.50	1.20

\*Significantly different from solvent p 0.05

\*\*Slope not significantly different from zero p = 0.05